RESPIRATORY METABOLISM OF AMPHIBIAN GASTRULA EXPLANTS

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Chemical embryologists suppose that familiar differences between the *morphological* behavior of the dorsal side of an amphibian gastrula and that of its ventral side are associated with causally significant differences in their *metabolic* behavior. But numerous attempts to confirm this plausible hypothesis have yielded results that are somewhat disappointing. Several items contribute to this undesirable state of affairs: crucial technical difficulties in handling gastrula explants are only now coming to be explicitly recognized; there is a seeming reluctance among chemical embryologists to repeat the work of others, so that probably unwarranted conclusions are currently accepted; and, worst of all, even the most carefully obtained results have not been suggestive of really fruitful explanatory hypotheses.

Nevertheless, there seems to be open no course other than to continue piecemeal the biochemical exploration of gastrulae in the hope that useful information will be obtained. In accordance with this view, we have (a) reinvestigated the confused question whether or not there is a dorso-ventral differential in respiratory rate in amphibian gastrulae, (b) studied the respiratory response of gastrulae tissues to the action of metabolic poisons that suppress gastrulation, in the expectation that dorsal and ventral explants might respond differently, and (c) tried to confirm the interesting report of Boell, Needham and Rogers (1939) that anaerobic carbon dioxide production of dorsal explants is about triple that of ventral ones.

Methods

Explants

Rana pipiens embryos at Stage 10 (dorsal lip; Shumway, 1940) were dissected in full-strength Holtfreter's solution with the aid of glass needles. Each dissected gastrula yielded four explants (Fig. 1); dorsal-left (D,L), dorsal-right (D,R), ventral-left (V,L) and ventral-right (V,R). A serious attempt was made to obtain explants symmetrically located with respect to the dorso-ventral and animal-vegetal axes. Loose yolk cells were removed before and after the explants were healed.

Gas exchange measurements

Measurements of respiratory rate (QO_2: μl oxygen/mg. dry weight of tissue hour at 22° C.) were made with the help of silicon-coated cylindrical Cartesian divers (Holter, 1943) whose average total volume was about 17 μl. Each diver contained the following items:

Two explants of the same class: (D,L) or (D,R) or (V,L) or (V,R)
Medium for explants (full-strength Holtfreter's, no bicarbonate): 0.75 μl
NaOH seal: 1.25 μl
Oil seal: 0.9 μl
Mouth seal: 2.5 μl

With these divers, and employing as a test system autoxidizing cysteine, oxidation rates varying between 0.01 and 0.05 μl/hour could be estimated with a standard deviation of 0.002 (25 experiments).

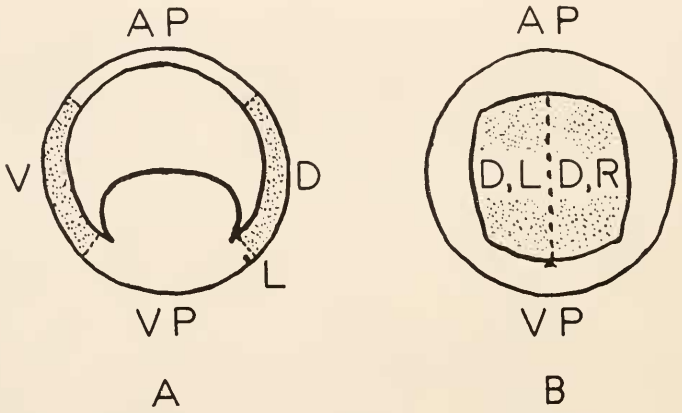

FIGURE 1. A. Diagram of medial sagittal section of a Stage 10 gastrula. Stippled areas show positions from which dorsal (D) and ventral (V) explants were taken. B. Dorsal view of Stage 10 gastrula, showing how dorsal explant is cut into left (D,L) and right (D,R) parts. The ventral explant is similarly treated. Other abbreviations: AP = animal pole; VP = vegetal pole; L = dorsal lip.

Using the same divers, anaerobic carbon dioxide production ($Q_{an}CO_2$: μl carbon dioxide produced/mg. dry weight of tissue/hour at 22° C.) was measured by a method essentially that of Boell, Needham and Rogers (1939). Each diver contained:

Two explants of the same class
Medium for explants (0.03 *M* bicarbonate in full-strength Holtfreter's solution): 0.75 μl
Oil seal: 0.9 μl
Mouth seal: 3.4 μl

Anaerobiosis was insured by the following procedure. After introducing explants into a diver (see below) it was mounted in such a way that it was held upright in a spring clamp with its mouth about two centimeters below the surface of some 95% flotation medium (without taurocholate) contained in a glass cylinder mounted on a vertically-moving rack and pinion (Fig. 2). A stream of gas (95 parts N_2: 5 parts CO_2, previously de-oxygenated over hot copper and washed with water) was then led into the diver with a capillary pipette (as shown) for at least one minute. The diver was then moved along to a second pipette which was used to

place the oil seal. The space above the oil seal was next re-gassed for about fifteen seconds, and the diver moved along to a third pipette, which was used to suck out gas from above the oil seal until by test the diver barely stayed afloat in the medium in the cylinder. This latter procedure automatically introduced a mouth seal of the proper volume.[1]

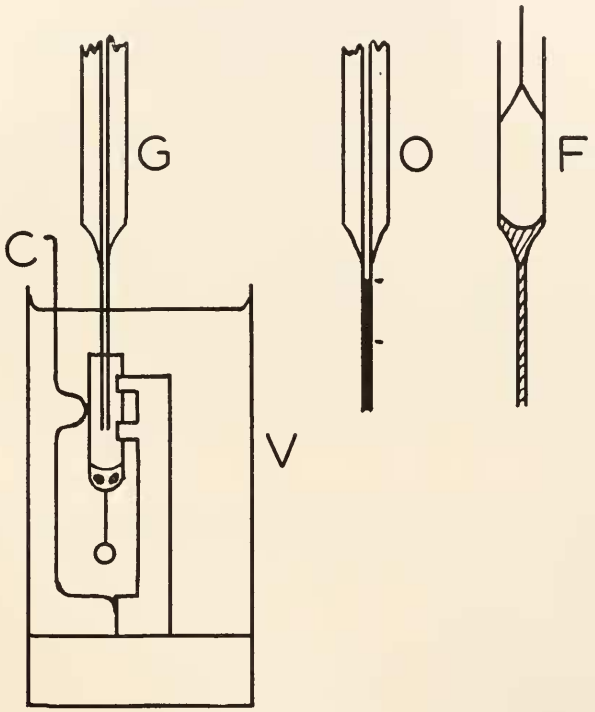

FIGURE 2. Diagram of apparatus used to fill divers anaerobically (not to scale). Diver with two explants shown held by spring clamp (C) in glass vessel (V) filled with flotation medium. Gas pipette (G) in position to gas diver. (O), pipette filled with oil, ready to place oil seal. Two marks represent calibration marks. (F), braking pipette partly filled with flotation medium, ready to insert into diver and remove gas from above oil seal, thus placing mouth seal.

We believe that nearly complete anaerobiosis is thus achieved. For example, a cysteine solution was prepared, samples of which autoxidized under iron catalysis in air-filled divers at the rate of 0.105 μl/hour, whereas control samples in anaerobic divers exhibited a gas uptake of only 0.0009 ± 0.0018 μl/hour (3 experiments). Obviously, oxygen uptake in the anaerobic divers is not significantly different from zero.

When the divers were filled, they were at once transferred to the diver flotation vessels in a constant temperature bath (22° C.) and equilibrated for at least one

[1] This apparatus was kindly lent to us by Dr. L. C. Sze. He used it by filling the cylinder with distilled water kept anaerobic by bubbling the $N_2 : CO_2$ mixture through it. The practice of using flotation medium, in which oxygen is negligibly soluble, is our own innovation.

half hour before readings were begun. Readings were generally made for two hours at intervals of from 10 to 15 minutes.

Loading explants into divers, removing and weighing

Explants were easily and safely loaded into the divers in the following manner. Each diver was filled over-full with the proper explant medium. Explants were then transferred with a braking pipette into the mouth of the diver. When they had sunk to the bottom of the diver, excess medium was removed with a fine capillary pipette.

Following the readings, each diver was removed from its flotation vessel, washed off on the outside with distilled water, and all its seals removed by flushing its neck copiously with distilled water. It was then filled completely with distilled water, and inverted with its mouth under the water contained in a small glass dish until the explants fell slowly out. From the distilled water dish the explants were transferred with a braking pipette, along with about 5 μl water, to small pre-weighed bits of cigaret paper. After drying 30 minutes to overnight at 100° C., the papers and explants were re-weighed to 1 μg on a quartz helix microbalance. On the average, two explants weighed approximately 100 μg.

None of these procedures entailed any danger that the explants would be injured or destroyed by rough handling or by contact with an air-water interface.

Inhibitor solutions

Inhibitor solutions (made up in full-strength Holtfreter's without bicarbonate) employed in the experiments following had different pH values:

Sodium azide: 6.3–6.4
Potassium cyanide: 9.8
Sodium malonate: 7.1–7.3
p-chloromercuribenzoate: 7.9–9.8
Sodium fluoride: 5.8–5.9
2,4 dinitrophenol: 5.0–5.1

Control explants were run in Holtfreter's adjusted with NaOH or HCl to the pH of the corresponding inhibitor solutions. A survey of the results will show that respiratory activity of explants was the same in all these control solutions as in plain Holtfreter's. Furthermore, whole gastrulae develop normally in all of them.

Throughout the inhibitor studies reported in this paper, for each experimental (control) explant used, there was a corresponding control (experimental) explant *from the same gastrula.*

To maintain cyanide concentrations around the explants in the divers (pH 9.8) it was necessary to add cyanide to the NaOH seal as follows:

KCN around explants	*KCN in NaOH seal*
0.0001 *M*	0.09 *M*
0.0005 *M*	0.44 *M*

(*cf.* Umbreit, Burris and Stauffer, 1945; p. 45 ff.).

Results

Respiration in full-strength Holtfreter's solution

In Holtfreter's solution, which for the purpose of these investigations we shall consider to constitute a relatively normal environment for explants, lateral halves of dorsal explants respire at about the same rate ($P > 0.05$) :[2]

$$(1) \quad QO_2\ (D,L) = 0.30 \pm 0.08 \qquad (20)^3$$

$$(2) \quad QO_2\ (D,R) = 0.29 \pm 0.05 \qquad (14)$$

A similar result has been obtained for lateral halves of ventral explants ($P > 0.05$) :

$$(3) \quad QO_2\ (V,L) = 0.25 \pm 0.05 \qquad (19)$$

$$(4) \quad QO_2\ (V,R) = 0.25 \pm 0.08 \qquad (14)$$

A further result of these measurements is that we have confirmed the reports of those investigators who have found a dorso-ventral gradient in respiration with respect to dry weight, for dorsal explants respire at a significantly higher rate than ventral explants [4] ($P < 0.001$) :

$$(5) \quad \frac{QO_2\ (D,L \text{ and } R)}{QO_2\ (V,L \text{ and } R)} = 1.2 \pm 0.2 \qquad (31)$$

It is not likely, however, that this finding will provide much basis for speculation about the relations of respiratory rate to gastrulation, for it seems that when respiratory rates are expressed in terms of some non-yolk reference component, the respiratory rate of dorsal explants comes out to be identical with that of ventral ones. We do not discuss this question in detail. A forthcoming paper by Sze carefully analyzes the whole problem of respiratory gradients in amphibian gastrulae, and his results shed much light on current disagreements. Our findings are quite in line with his. It thus appears that earlier expectations of discovering embryologically significant differences in the respiratory rates of various gastrula-parts under normal conditions are coming to be less and less warranted by new results. Nevertheless there remains the possibility that differential respiratory responses to less physiological environments may yet be obtained. To this question we not turn.

Respiration in sodium azide (NaN_3)

Sodium azide is thought to act upon biological systems by inactivating the cytochrome oxidase system as cyanide does, or by interfering with the action of some enzyme systems involved in anaerobic phosphate transfer. Azide is a powerful inhibitor of gastrulation in amphibian embryos: both Barnes (1944) and Spiegelman and Moog (1945) have reported the failure of *Rana pipiens* embryos to gastru-

[2] For calculating P-values, Student's t-test has been used throughout.

[3] Parenthesized numerals to the extreme right of equations (1)—(65) indicate the number of experiments performed.

[4] In considering quotients such as (5) it may be of help to know that throughout this paper they are calculated only from the results of experiments in which for each dorsal (ventral) explant used there was a corresponding ventral (dorsal) explant *from the same gastrula.*

late in weak concentrations of azide; and our results confirm theirs, for gastrulae placed at Stage 10 in 0.0005–0.001 M sodium azide do not develop past Stage 10^{+}. Barnes showed also that the respiratory rate of whole gastrulae is sharply reduced (80–90%) in 0.01 M azide. But the respiration of gastrula tissues is sensitive even to weaker concentrations of azide.

In 0.0005 M azide, for example, dorsal explants respire at a significantly lower rate than their controls ($P < 0.01$):

(6) QO_2 (D,R) 0.0005 M $NaN_3 = 0.08 \pm 0$ (3)

(7) QO_2 (D,L) control $= 0.23 \pm 0.08$ (3)

There is a similar reduction of the respiration of ventral explants ($P < 0.001$):

(8) QO_2 (V,R) 0.0005 M $NaN_3 = 0.06 \pm 0.02$ (3)

(9) QO_2 (V,L) control $= 0.20 \pm 0.05$ (3)

But there is no indication that the relative rates of respiration of dorsal and ventral tissues are changed by this concentration of azide ($P > 0.05$):

(10) $$\frac{QO_2 \text{ (D,R) } NaN_3}{QO_2 \text{ (V,R) } NaN_3} = 1.4 \pm 0.2$$ (3)

(11) $$\frac{QO_2 \text{ (D,L) control}}{QO_2 \text{ (V,L) control}} = 1.1 \pm 0.1$$ (3)

Analogous results have been obtained with 0.001 M azide. The respiration of dorsal explants is greatly reduced ($P < 0.001$):

(12) QO_2 (D,R) 0.001 M $NaN_3 = 0.09 \pm 0.02$ (3)

(13) QO_2 (D,L) control $= 0.32 \pm 0.04$ (3)

and so is that of ventral explants ($P < 0.001$):

(14) QO_2 (V,R) 0.001 M $NaN_3 = 0.10 \pm 0.02$ (3)

(15) QO_2 (V,L) control $= 0.29 \pm 0.04$ (3)

But, although statistical analysis indicates a probably significant alteration in the relative rates of respiration of dorsal and ventral explants ($P < 0.05$):

(16) $$\frac{QO_2 \text{ (D,R) } NaN_3}{QO_2 \text{ (V,R) } NaN_3} = 0.87 \pm 0.22$$ (3)

(17) $$\frac{QO_2 \text{ (D,L) control}}{QO_2 \text{ (V,L) control}} = 1.1 \pm 0.2$$ (3)

in view of the results obtained with 0.0005 M azide ((6)–(11)) we do not consider this established.

To sum up these results with azide, it appears that if gastrulation of *Rana pipiens* embryos is blocked by a specific action of azide, then either this action is not localized in dorsal tissues or else it is one that respiratory measurements do not reveal.

Respiration in potassium cyanide (KCN)

Cyanide, which inhibits respiration in biological systems by blocking cytochrome oxidase electron transfer, is also a strong suppressor of morphogenetic movements in amphibian embryos. Barnes (1944), for example, found that early gastrulae placed in 0.001–0.002 *M* KCN developed only into mid-gastrulae; 0.0001 *M* KCN only slowed gastrulation. Spiegelman and Moog (1945) partially confirmed Barnes's results: in 0.001 *M* KCN, embryos immersed at Stage 10 developed into late gastrulae (Stage 12); while in 0.005 *M* KCN, embryos immersed at Stage 10 remained at that stage. Our experiments indicate variable results with 0.0005 *M* KCN ranging all the way from complete inhibition of invagination to slight slowing only; while with 0.005 *M* KCN, early gastrulae never get past Stage 11.

Barnes also made some measurements of the effect of KCN on the oxygen uptake of whole embryos. She found a maximal effect at concentrations of 0.001 *M*, at which respiration of gastrulae was inhibited about 98%, with graded lesser effects at lower concentrations.

Our results are partly confirmatory of hers. In 0.0001 *M* KCN the respiration of dorsal explants is reduced by a probably significant amount ($P < 0.05$):

(18) QO_2 (D,R) 0.0001 *M* KCN $= 0.20 \pm 0.02$ (2)

(19) QO_2 (D,L) control $= 0.31 \pm 0.05$ (2)

There is a similar effect on ventral explants ($P = 0.01$):

(20) QO_2 (V,R) 0.0001 *M* KCN $= 0.15 \pm 0$ (2)

(21) QO_2 (V,L) control $= 0.27 \pm 0.04$ (2)

Statistically speaking, the effect is probably differential ($P < 0.05$):

(22) $$\frac{QO_2 \text{ (D,R) KCN}}{QO_2 \text{ (V,R) KCN}} = 1.4 \pm 0.1 \quad (2)$$

(23) $$\frac{QO_2 \text{ (D,L) control}}{QO_2 \text{ (V,L) control}} = 1.1 \pm 0.1 \quad (2)$$

But in view of the variable results on whole embryos with this extremely weak solution, and in view of the fact that stronger solutions give no such effect, we do not regard this as established by our data. In somewhat stronger (0.0005 *M*) solutions of cyanide, the respiration of explants is reduced a little more for both dorsal (P barely over 0.01) and ventral ($P < 0.01$) explants:

(24) QO_2 (D,R) 0.0005 *M* KCN $= 0.10 \pm 0.02$ (2)

(25) QO_2 (D,L) control $= 0.26 \pm 0.04$ (2)

(26) QO_2 (V,R) 0.0005 *M* KCN $= 0.09 \pm 0.01$ (2)

(27) QO_2 (V,L) control $= 0.18 \pm 0.02$ (2)

But this effect is not a differential one ($P > 0.05$):

$$(28)\quad \frac{QO_2\ (D,R)\ KCN}{QO_2\ (V,R)\ KCN} = 1.1 \pm 0.1 \qquad (2)$$

$$(29)\quad \frac{QO_2\ (D,L)\ control}{QO_2\ (V,L)\ control} = 1.4 \pm 0.1 \qquad (2)$$

Summarizing: Although it is true that cyanide depresses the respiration of gastrula explants (as some function of its concentration), there is little indication from the respiratory effects that its action in blocking gastrulation is an anatomically localized one.

Respiration in sodium malonate (mal)

Another inhibitor of cellular oxidations is malonic acid which inhibits the conversion by succinic acid dehydrogenase of succinic acid to fumaric acid in the Krebs cycle. So far as we are aware, the only report of the effect of malonate on amphibian gastrula tissue is a report by Brachet and Rapkine (1939) to the effect that dorsal fragments develop normally in 0.025 *M* malonate. Precisely what this means is not clear.

But in new experiments, we have found that whole embryos placed at Stage 10 in 0.04 *M* sodium malonate do not develop past Stage 11^+. Furthermore, the respiratory activities of both dorsal and ventral explants are curtailed significantly ($P < 0.01$ in both cases) by malonate in this concentration:

$$(30)\quad QO_2\ (D,R)\ 0.04\ M\ mal = 0.13 \pm 0.01 \qquad (2)$$

$$(31)\quad QO_2\ (D,L)\ control = 0.32 \pm 0.03 \qquad (2)$$

$$(32)\quad QO_2\ (V,R)\ 0.04\ M\ mal = 0.11 \pm 0.01 \qquad (2)$$

$$(33)\quad QO_2\ (V,L)\ control = 0.26 \pm 0.03 \qquad (2)$$

But this curtailment is not a differential one ($P > 0.05$):

$$(34)\quad \frac{QO_2\ (D,R)\ mal}{QO_2\ (V,R)\ mal} = 1.2 \pm 0.1 \qquad (2)$$

$$(35)\quad \frac{QO_2\ (D,L)\ control}{QO_2\ (V,L)\ control} = 1.2 \pm 0.03 \qquad (2)$$

In summary: like other compounds which block dehydrogenase activities (cyanide, azide), malonate reduces the rate of oxygen utilization of gastrula tissues; but, again, the pattern of inhibition furnishes no clue (beyond some very general ones) to the manner in which malonate blocks gastrulation movements. We turn now to a different class of inhibitors.

Respiration in p-chloromercuribenzoic acid (HgB)

Barron and Singer (1945) and Singer and Barron (1945) have shown that p-chloromercuribenzoate is a powerful inhibitor of a large number of enzymes in-

volved in carbohydrate, protein, and fat metabolism. This reagent is thought to act by combining with all available —SH groups in native protein, unlike, for example, ferricyanide which acts only on those —SH groups close enough to form disulfides, or iodoacetate, which in low concentrations combines incompletely with even free —SH groups in native protein.

It is interesting that although 0.0001 *M* p-chloromercuribenzoate is able to prevent further development of gastrulae immersed in it at Stage 10, it has no respiratory effect whatsoever at this concentration ($P > 0.05$ in all cases):

(36) QO_2 (D,R) 0.0001 *M* HgB $= 0.31 \pm 0.06$ (2)

(37) QO_2 (D,L) control $= 0.36 \pm 0.08$ (2)

(38) QO_2 (V,R) 0.0001 *M* HgB $= 0.26 \pm 0.09$ (2)

(39) QO_2 (V,L) control $= 0.26 \pm 0.08$ (2)

(40) $$\frac{QO_2 \text{ (D,R) HgB}}{QO_2 \text{ (V,R) HgB}} = 1.3 \pm 0.2$$ (2)

(41) $$\frac{QO_2 \text{ (D,L) control}}{QO_2 \text{ (V,L) control}} = 1.4 \pm 0.1$$ (2)

Gastrulae placed at Stage 10 in 0.001 *M* p-chloromercuribenzoate do not develop further. But at *this* concentration the respiration of dorsal explants is sharply and significantly reduced ($P < 0.05$), and so is that of ventral explants ($P < 0.01$):

(42) QO_2 (D,R) 0.001 *M* HgB $= 0.10 \pm 0.02$ (2)

(43) QO_2 (D,L) control $= 0.28 \pm 0.05$ (2)

(44) QO_2 (V,R) 0.001 *M* HgB $= 0.08 \pm 0.01$ (2)

(45) QO_2 (V,L) control $= 0.23 \pm 0$ (2)

However, there is no differential effect ($P > 0.05$) of this —SH inhibitor on the respiration of dorsal and ventral explants:

(46) $$\frac{QO_2 \text{ (D,R) HgB}}{QO_2 \text{ (V,R) HgB}} = 1.2 \pm 0.1$$ (2)

(47) $$\frac{QO_2 \text{ (D,L) control}}{QO_2 \text{ (V,L) control}} = 1.2 \pm 0.2$$ (2)

Summarizing: p-chloromercuribenzoate will reduce the respiratory exchange of gastrula explants, but the effect upon dorsal and ventral explants is similar. Furthermore, it will block gastrulation in whole embryos. But the effect upon respiration and that upon gastrulation are not associated in any simple manner, for gastrulation may be completely blocked in the total absence of any effect upon the respiration.

Respiration in sodium fluoride (NaF)

Sodium fluoride is known to inhibit the conversion of 2-phosphoglyceric acid into phospho-enol-pyruvic acid by enolase (an enzyme requiring Mg ions) presumably by forming a complex magnesium fluorophosphate in the presence of inorganic phosphate (Baldwin, 1948)—thus preventing the anaerobic production of energy-rich phosphate bonds. In proper concentrations, it is well known to inhibit developmental processes. For example, developing amphibian embryos have been found to be checked by 0.02 *M* NaF but not by 0.005 *M* (Pomerat and Haringa, 1939; *R. pipiens*), and not by 0.01 *M* (Brachet, 1950; *R. fusca*). We have found that in 0.02 *M* NaF gastrulae immersed at Stage 10 do not develop past Stage 14; while in 0.04 *M* NaF they do not develop past Stage 11^+. We have not investigated the effects of weaker concentrations.

But we have not confirmed Brachet's statement (1950, p. 327) that the respiration of amphibian embryos (*R. fusca*) is not fluoride-sensitive; for 0.02 *M* NaF significantly reduces the respiration of both dorsal ($P < 0.01$) and ventral ($P < 0.001$) explants:

$$(48)\quad QO_2\ (D,R)\ 0.02\ M\ NaF = 0.19 \pm 0.08 \qquad (3)$$

$$(49)\quad QO_2\ (D,L)\ control = 0.32 \pm 0.04 \qquad (3)$$

$$(50)\quad QO_2\ (V,R)\ 0.02\ M\ NaF = 0.14 \pm 0.02 \qquad (3)$$

$$(51)\quad QO_2\ (V,L)\ control = 0.24 \pm 0.03 \qquad (3)$$

As a result of this treatment, however, there was no significant change in the relative respiratory rates of dorsal and ventral explants ($P > 0.05$):

$$(52)\quad \frac{QO_2\ (D,R)\ NaF}{QO_2\ (V,R)\ NaF} = 1.3 \pm 0.4 \qquad (3)$$

$$(53)\quad \frac{QO_2\ (D,L)\ control}{QO_2\ (V,L)\ control} = 1.4 \pm 0.1 \qquad (3)$$

Summarizing: The pattern of fluoride inhibition of respiration of gastrula explants is much like that of other types of metabolic poisons: dorsal and ventral explants are similarly affected.

Respiration in 2,4 dinitrophenol (DNP)

We cannot confirm Andreassi's (1942) report (not available to us; see Brachet, 1950, p. 321) that dinitrophenol accelerates amphibian development; rather, our results support Dawson's (1938) results. Whole gastrulae placed at Stage 10 in 9.5×10^{-6} *M* 2,4 dinitrophenol (α-dinitrophenol; hereafter, DNP) do not develop past Stage 11. In spite of this, however, the tissues of such gastrulae respire at more than twice their normal rate. The respiration of dorsal explants is significantly increased ($P < 0.001$):

$$(54)\quad QO_2\ (D,R)\ 9.5 \times 10^{-6}\ M\ DNP = 0.66 \pm 0.21 \qquad (4)$$

$$(55)\quad QO_2\ (D,L)\ control = 0.31 \pm 0.09 \qquad (4)$$

There is a similar significant increase in the respiration of ventral explants in DNP ($P < 0.001$):

$$(56)\quad QO_2\ (V,R)\ 9.5 \times 10^{-6}\ M\ DNP = 0.53 \pm 0.06 \qquad (4)$$

$$(57)\quad QO_2\ (V,L)\ control = 0.24 \pm 0.08 \qquad (4)$$

But the relative rates of dorsal and ventral explants are not significantly different ($P > 0.05$):

$$(58)\quad \frac{QO_2\ (D,R)\ DNP}{QO_2\ (V,R)\ DNP} = 1.2 \pm 0.2 \qquad (4)$$

$$(59)\quad \frac{QO_2\ (D,L)\ control}{QO_2\ (V,L)\ control} = 1.3 \pm 0.1 \qquad (4)$$

In summary: this particular dinitrophenol behaves like other inhibitors of development in failing to act differentially upon the respiratory metabolism of dorsal and ventral explants. Its effect also provides another instance of the ease with which respiratory activity may be dissociated from morphogenetic activity. We now abandon the attempt to affect oxidative metabolism of dorsal and ventral explants differentially and turn to the question whether or not they differ in their anaerobic metabolism.

Anaerobic carbon dioxide production

Boell, Needham and Rogers (1939) reported that under anaerobiosis the CO_2-production of dorsal pieces of amphibian gastrulae is about three times greater than that of ventral pieces. Unfortunately, they believe also that there was an exactly analogous differential in anaerobic ammonia production, and their final values for CO_2-output were arrived at by taking into account the possibility that some 70% of the CO_2 produced was masked by ammonia, which combines with lactic acid produced anaerobically and hence prevents this acid from liberating CO_2 from the bicarbonate medium in which the explants were suspended. But we have recently (Gregg and Ornstein, 1952) tried to cast serious doubt on the reliability of their results: for in early gastrulae we were able to find no significant amount of ammonia produced by gastrula tissues—aerobically or anaerobically—and we have accounted for their ammonia values as artifacts. Consequently, we have felt it necessary to re-investigate the question of anaerobic CO_2-production by gastrula explants—and once again we have failed to confirm their findings.

Dorsal explants of gastrulae do, as they believed, produce CO_2 under anaerobic conditions:

$$(60)\quad QanCO_2\ (D,L\ and\ R) = 0.11 \pm 0.04 \qquad (14)$$

So do ventral explants, though at only a slightly lesser rate:

$$(61)\quad QanCO_2\ (V,L\ and\ R) = 0.10 \pm 0.05 \qquad (13)$$

This small difference between dorsal and ventral pieces, the ratio being:

$$(62)\quad \frac{\mathrm{QanCO_2\ (D,L\ and\ R)}}{\mathrm{QanCO_2\ (V,L\ and\ R)}} = 1.1 \pm 0.3 \qquad (13)$$

is not statistically significant ($P > 0.05$): thus we must conclude from these results that there is no striking difference between the anaerobic CO_2 output of these two parts of the amphibian gastrula.

Our results have been independently confirmed by Miss Adele Kostellow. With her kind permission her results (at 25° C.) are summarized below:

$$\mathrm{QanCO_2\ (D,L\ and\ R)} = 0.12 \pm 0.04 \qquad (8)$$

$$\mathrm{QanCO_2\ (V,L\ and\ R)} = 0.10 \pm 0.04 \qquad (8)$$

$$\frac{\mathrm{QanCO_2\ (D,L\ and\ R)}}{\mathrm{QanCO_2\ (V,L\ and\ R)}} = 1.4 \pm 0.7 \qquad (8)$$

As in our experiments, the difference between dorsal and ventral explants is not significant ($P > 0.05$). The high standard deviation (0.7) of her ratios is due to the presence of one experiment in which there was a ratio of 3. If this experiment is deleted, the standard deviation is reduced to 0.2, and the value of the ratio becomes 1.1.

It is not clear to us why our results are at variance with those of Boell *et al.* Perhaps their (apparently unwarranted) correction of CO_2-values by ammonia-values is partly to blame; but among other items which should be considered (and about which information is lacking) are: species-differences (they used *R. temporaria* and *Triton alpestris*); relative stages of gastrulae from which explants were made (we used Stage 10, they used more advanced stages, apparently); duration of anaerobiosis (about five hours for them, about two hours for us); and condition of the explants in the divers (never cytolyzed by interfaces in our experiments; apparently sometimes cytolyzed in theirs; *cf.*, their remarks, p. 353, and Brachet, 1950).

About the last point (explant injury) some comment should be made. In our experiments, the explants were suspended in Holtfreter's solution containing 0.03 *M* bicarbonate (pH after gassing: ca. 7.4), and in this solution the surface-coat of the explants tended to corrode slightly. Visual inspection revealed no apparent cytolysis, but as a confirmatory check on the normality of explants in this solution we obtained their oxygen uptakes, with the result that dorsal explants do indeed respire at a rate significantly different from that of dorsal explants in Holtfreter's without bicarbonate ($P < 0.001$; *cf.* (63) with (1) and (2)), while such ventral explants do not ($P > 0.05$; *cf.* (64) with (3) and (4)).

$$(63)\quad \mathrm{QO_2\ (D,L)\ 0.03\ \textit{M}\ HCO_3} = 0.27 \pm 0.07 \qquad (7)$$

$$(64)\quad \mathrm{QO_2\ (V,L)\ 0.03\ \textit{M}\ HCO_3} = 0.25 \pm 0.06 \qquad (7)$$

But, although the relative respiratory rate of dorsal and ventral tissues:

$$(65)\quad \frac{\mathrm{QO_2\ (D,L)\ HCO_3}}{\mathrm{QO_2\ (V,L)\ HCO_3}} = 1.1 \pm 0.2 \qquad (7)$$

is changed slightly by bicarbonate ((P < 0.001); (65) compared with (5)), the change is so small that we do not consider it as indicating any crucial actual damage to the tissues.

Summarizing: Contrary to the report of Boell, Needham and Rogers (1939), dorsal and ventral explants do not produce carbon dioxide anaerobically at significantly different rates.

Summary

1. In full-strength Holtfreter's solution, dorsal explants from *Rana pipiens* gastrulae respire some 20% faster than ventral explants from the same gastrulae (with respect to dry weight). Lateral halves of dorsal explants respire at the same rate; and lateral halves of ventral explants respire at the same rate.

2. Azide, cyanide, malonate, p-chloromercuribenzoate, fluoride and 2,4 dinitrophenol in low concentrations all either completely block or greatly retard the gastrulation movements of *Rana pipiens* embryos.

3. Azide, cyanide, malonate, p-chloromercuribenzoate and fluoride all sharply reduce the respiratory rate of both dorsal and ventral explants from 29% (0.0001 *M* KCN) to 72% (0.001 *M* NaN_3).

4. 2,4 dinitrophenol (9.5×10^{-6} *M*) more than doubles the respiratory rate of both dorsal and ventral explants.

5. If these inhibitors block the movements of gastrulation by differential effects upon dorsal and ventral tissues, then these effects are not of such a nature as to be revealed by respiratory measurements—for the relative respiratory rates of dorsal and ventral tissues exposed to inhibitors are the same as those of controls in Holtfreter's solution.

6. Respiratory activity is not linked in any simple way with the movements of gastrulation, for compounds that reduce the former may have no effect upon the latter (0.0005 *M* KCN), and compounds that may completely block the latter have no effect upon the former (0.0001 *M* p-chloromercuribenzoate).

7. Dorsal and ventral explants of *Rana pipiens* gastrulae produce carbon dioxide anaerobically, but at rates that are not significantly different.

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